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Production of serum amyloid A and C-reactive protein by HepG2 cells stimulated with combinations of cytokines or monocyte conditioned media: the effects of prednisolone

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SUMMARY

The hepatic production of the acute phase proteins in response to inflammatory cytokines, and the interaction of corticosteroids within this response, has been the subject of considerable recent research. In this study we have examined the effects of the corticosteroid prednisolone on the production of IL-1α and IL-1β by lipopolysaccharide (LPS)-stimulated monocytes, and the ability of the monocyte conditioned media (MOCM) obtained under these conditions to induce human hepatoma HepG2 cells to produce serum amyloid A (SAA) and C-reactive protein (CRP). We also examined the production of SAA and CRP by HepG2 cells exposed to different combinations and concentrations of recombinant human (rh) IL-1α, rhIL-1β, rhIL-6, recombinant human tumour necrosis factor-alpha (rhTNF-α) and prednisolone. The findings indicate: (i) prednisolone substantially inhibits the production of both IL-1α and IL-1β by LPS-stimulated monocytes. The MOCM from prednisolone-treated monocytes induced less SAA and CRP production by HepG2 cells; (ii) IL-1α and IL-1β both induced CRP and SAA synthesis by HepG2 cells, but only in the presence of IL-6. IL-1β was the more potent inducer for SAA production, but for CRP production IL-1α and IL-1β were equivalent; (iii) prednisolone enhances the production of SAA by HepG2 cells, but does not enhance the production of CRP; (iv) TNF-α in the presence or absence of IL-6 and/or prednisolone did not induce the production of SAA or CRP by HepG2 cells. These findings offer a tenable solution to a disparate production of SAA compared with CRP in corticosteroid-treated cystic fibrosis (CF) patients.

Keywords serum amyloid A C-reactive protein corticosteroids cytokines HepG2

INTRODUCTION

Acute phase proteins are synthesized by the liver as part of a non-specific response to tissue damage or infection [1]. The purpose of the acute phase response is to confine the source of inflammation and limit autolytic damage by phagocytic cells [2]. Regulation of the synthesis of acute phase proteins is modulated by a very complex network of cytokines which can act independently or in concert with each other and with endocrine hormones and glucocorticoids to inhibit or stimulate the production of acute phase proteins [3]. The major cytokines involved are IL-1, IL-6, tumour necrosis factor (TNF), and leukaemia inhibitory factor (LIF) [4]. Recent observations have shown that the activity in promoting the production of the acute phase reactants, as well as the binding characteristics of IL-1α and IL-1β are not identical. IL-1α and IL-1β have only a 26% homologous amino acid sequence [5], and the two IL-1 genes have very little in common [6]. The IL-1β gene was shown to be clearly dominant in human monocytes, and to have a strong enhancer allowing high levels of expression [6], whereas IL-1α was primarily produced by keratinocytes [7] and T cells [8].

The receptor expression and cellular distribution for IL-1α and IL-1β also differ significantly. Scapigliati *et al.* [9] demonstrated that murine EL4-6.1 thymoma cells possessed more than 22 000 receptors per cell for IL-1α (Kd 1·0 nM), but less than 3000 receptors for IL-1β (Kd 0·36 nM). Human B lymphoma Raji cells, however, have nearly eight times as many receptors for IL-1β (2400 receptors per cell, Kd 2·2 nM) as for IL-1α (316 receptors per cell, Kd 0·13 nM). Scapigliati also demonstrated that unlabelled IL-1β was able to competitively displace both radiolabelled IL-1α and IL-1β from Raji cells, while unlabelled IL-1α was unable to displace radiolabelled IL-1β. The receptor which predominates on T cells and preferentially binds IL-1α has a molecular weight of 80 kD, and the B cell receptor, which binds only IL-1β, has a molecular weight of 68 kD. Ghiara *et al.*

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[10] recently demonstrated that HepG2 cells express both receptors, approximately 1300 of the IL-1α binding receptors (Kd 0·18 nM) and 417 of the IL-1β receptors (Kd 0·29 nM).

The acute phase proteins exhibit different magnitudes of increase following stimulus [11]. The plasma concentrations of C-reactive protein (CRP) and serum amyloid A (SAA) may increase as much as several thousand-fold over normal physiological concentrations [12] with SAA reaching concentrations as high as 1 mg/ml during acute inflammation [1]. Plasma CRP levels have been used as an aid in diagnosis of both primary conditions and superimposed infections, and as a measure of disease activity in patients suffering from rheumatoid arthritis and other inflammatory disorders, malignancies, etc. [1, 11]. SAA level has similar clinical value, and in some instances has been shown to be a more sensitive indicator of inflammation than CRP [13–16]. Plasma levels of SAA and CRP correlate with pulmonary inflammation secondary to lung infections in cystic fibrosis (CF) patients [13, 16]. We recently reported an apparent disparate production of SAA compared with CRP in CF patients receiving systemic steroidal anti-inflammatory therapy when they developed *Pseudomonas aeruginosa* lung infections, wherein the ratio of SAA to CRP production was significantly increased (three-fold) when compared with patients not receiving corticosteroid therapy [16].

These observations prompted us to examine the effects of prednisolone on the production of SAA and CRP in HepG2 cells stimulated with IL-1α, IL-1β or TNF-α in combination with each other and/or IL-6. The effect of prednisolone on the production of IL-1α and IL-1β by peripheral blood monocytes when stimulated with lipopolysaccharide (LPS) from *Escherichia coli* or with killed *Ps. aeruginosa* was examined. The ability of the monocyte conditioned media (MOCM) obtained under these conditions to induce HepG2 cells to produce SAA and CRP was also evaluated.

MATERIALS AND METHODS

Cytokines

Recombinant human (rh) IL-1α and rhIL-1β were obtained from the Biological Response Modifier Program, National Cancer Institute (Frederick, MD). rhIL-6 was a very kind gift from Drs L. May and P. Sehgal (Rockefeller University, New York, NY). rhTNF-α was from Genentech (South San Francisco, CA).

IL-1 MoAbs

All antibodies for IL-1α and IL-1β assays, as well as neutralizing antibodies for IL-1α and IL-1β, were very kind gifts from Dr John Kenney, Syntex Laboratories (Palo Alto, CA).

Cell culture

All cells were maintained in prepared RPMI 1640 medium (GIBCO, Grand Island, NY) (with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), 2 mM L-glutamine, penicillin/streptomycin, and fungizone (all from GIBCO)) and were incubated at 37°C in an atmosphere of 5% CO_2.

MOCM preparation

Fresh heparinized whole blood was obtained by venipuncture from healthy volunteers. The mononuclear cell fraction was separated by gradient centrifugation on Histopaque-1077 (Sigma, St Louis, MO). Monocytes were separated by adherence to plastic tissue culture flasks (Falcon, Becton Dickinson Labware, Lincoln Park, NJ) in prepared RPMI 1640 for 2 h. Non-adherent cells were removed and discarded. Adherent cells were washed twice, then placed in fresh medium, and incubated overnight at 37°C. These quiescent adherent cells were washed once more, placed in fresh medium, then stimulated with either 10 μg/ml LPS from *E. coli* (Difco, Detroit, MI) or a killed suspension of *Ps. aeruginosa*. After 48 h conditioned media were collected, centrifuged, filter sterilized, and stored at −20°C till used. Cells prepared in this manner appeared morphologically to be >95% monocytes. Viability of monocytes was routinely assessed by trypan blue exclusion after 48 h incubation with or without LPS, in the presence or absence of prednisolone, and was always greater than 90%.

Neutralization studies

MoAbs which have been shown to specifically neutralize IL-1α (ILA8-H12) or IL-1β (ILB1-H34.3) without cross-reacting with IL-2, IL-4, IL-6, or TNF [17, 18], or appropriate control material, were added to MOCM at 5 μg/ml, mixed thoroughly, and incubated at 4°C for 2 h. This amount of antibody was sufficient to neutralize up to 5 ng/ml IL-1α or IL-1β [18]. MOCM with both antibodies added at 5 μg/ml was also prepared in the same manner.

HepG2 cells

HepG2 human hepatoma cells were a gift from Dr Dean Tuma, Veterans Administration Hospital (Omaha, NE). Cells were passaged every 7 days, and were plated at approximately 5×10^4 cells/well in 24-well tissue culture plates (Falcon) or at 5×10^5 cells per 25 cm^2 tissue culture flask (Falcon). Hepatoma cells were stimulated with 20% MOCM or with various combinations of cytokines and/or corticosteroids on the fifth day after plating. After 48 h medium was removed and assayed for SAA and CRP, or was frozen at −20°C till assayed.

IL-1 assay

Vinyl assay plates (Costar, 2596, Cambridge, MA) were coated with either purified ILA9-H18.2 or ILB1-H6.81 diluted in PBS at 1·5 μg/well overnight at 4°C. After washing in 0·1% bovine serum albumin (BSA)/0·05% Thimerosal/PBS, non-specific binding sites were blocked by incubating for 1 h at room temperature with 5% non-fat dry milk/0·05% Thimerosal/PBS (200 μl/well). After washing, 50 μl MOCM or standards which had been appropriately diluted in 1% non-fat dry milk/0·05% Thimerosal/PBS were added with 50 μl of biotinylated ILA8-H12 (2 μg/ml) or ILB1- H67 (1 μg/ml), and incubated for 2 h at room temperature. After washing, 100 μl of a 1:3000 dilution of peroxidase conjugated strepavidin (Zymed Labs, South San Francisco, CA) were added and incubated for 1 h. After washing, 100 μl/well of peroxidase substrate solution were added and incubated in the dark for 30 min. Tetramethyl benzidine (TMB) substrate solution (Kirkegaard and Perry, Gaithersburg, MD) was used for the IL-1α assay, and orthophenylene diamine (OPD) substrate solution (1 mg/ml OPD/0·03% H_2O_2/0·1 M citrate buffer, pH 4·9) was used for the IL-1β assay. Absorbance was measured at 450 nm for the IL-1β assay, and at

630 nm for the IL-1α assay. Sensitivities were approximately 15 pg/ml [17, 18].

SAA assay

The SAA assay was a modification of the recently published assay of McDonald *et al.* [19]. Immulon II ELISA plates (Dynatech, Chantilly, VA) were coated with 1 μg/well purified anti-SAA MoAb 6B10 in pH 9·6 carbonate buffer for 1 h at 37°C. After washing with PBS/0·05% Tween-20, 90 μl (containing 0·4 μg) of an alkaline phosphatase-conjugated anti-SAA MoAb, 5G6 (specific for a separate, non-overlapping epitope of SAA) was added to the 6B10-coated well, immediately followed by test samples (10 μl) or appropriately diluted (in PBS/0·05% Tween-20) standards [20]. After a 1 h incubation at 37°C, the plate was washed with PBS/0·05% Tween-20, and 100 μl of 1 mg/ml alkaline phosphatase substrate (Sigma 104) dissolved in 10% diethanolamine/0·01% $MgCl_2.6H_2O$, pH 9·8, was added. After a 1-h incubation at 37°C, absorbance at 405 nm was measured. Sensitivity was approximately 2 ng/ml. The antibodies, purified SAA, standards, and the SAA assay are available from BioSource International (Camarillo, CA).

CRP assay

CRP in tissue culture fluid was assayed using the procedure described by Janssen *et al.* [21]. Vinyl assay plates (Costar, 2596, Cambridge, MA) were coated with rabbit anti-human CRP (Dako, Carpinteria, CA) diluted 1:500 in 0·1 M, pH 9·6 carbonate buffer (100 μl/well) for 45 min at 37°C. Plates were washed with 0·05 M Tris-HCl/0·3 M NaCl/0·05% Tween-20, pH 8·0 (CRP wash buffer), tissue culture supernatant or standards appropriately diluted in CRP wash buffer was added, and the plates were incubated an additional 45 min at 37°C. Plates were washed and horseradish peroxidase conjugated rabbit anti-human CRP (Dako) diluted 1:500 in CRP wash buffer was added, and incubated for 30 min at 37°C. Plates were washed, then developed as described previously for the IL-1β assay. Sensitivity of the assay was approximately 0·5 ng/ml.

RESULTS

Effect of prednisolone on production of IL-1α and IL-1β by monocytes

The effect of prednisolone on the ability of monocytes to produce IL-1α and IL-1β in our culture system is shown in Table 1. IL-1α and IL-1β were undetectable in MOCM in the absence of LPS stimulation, whether or not prednisolone was added. Prednisolone added to monocytes at a final concentration of 1·0 μM 6 h before stimulation with *E. coli* LPS reduced the production of IL-1α and IL-1β to 47% and 46% respectively when compared with monocytes stimulated with LPS in the absence of prednisolone. When prednisolone was added at the time of LPS stimulation, IL-1α and IL-1β production were 54% and 58% respectively, which indicated that the inhibition noted with prednisolone was not dependent on preincubation of the monocytes with prednisolone before LPS stimulation. Comparable results were obtained by stimulation of monocytes with killed *Ps. aeruginosa* (data not shown).

Effect of prednisolone on the ability of MOCM to induce HepG2 cells to produce SAA and CRP

Since there would be carry-over prednisolone in the conditioned media from monocytes treated with prednisolone, it was important to determine first the effect of prednisolone on the production of CRP and SAA by HepG2 cells stimulated with MOCM. In four experiments prednisolone (1·0 μM) was added along with MOCM obtained from LPS-stimulated monocytes which were not treated with prednisolone. The added prednisolone had no effect on CRP production, but induced HepG2 cells to produce a six-fold greater concentration of SAA. CRP concentrations were unaffected by prednisolone addition (11·3 ± 2·8 ng/ml in the absence of prednisolone, 11·8 ± 1·2 ng/ml in the presence of 1·0 μM prednisolone) while SAA production by HepG2 cells increased from 52 ng/ml (s.d. 16 ng/ml) to 312 ng/ml (s.d. 100 ng/ml) when prednisolone was added to 1·0 μM. In order to assure that prednisolone had no independent effect on the production of CRP and SAA by HepG2 cells, prednisolone was added without MOCM or with MOCM from

Table 1. Effect of prednisolone on the production of IL-1α and IL-1β by lipopolysaccharide (LPS)-stimulated monocytes*

	MOCM preparation				
	No LPS		10 μg/ml LPS		
	No prednisolone	1 μM prednisolone	No prednisolone	1 μM prednisolone t_{-6} h†	1 μM prednisolone t_0‡
IL-1α (pg/ml)	<15	<15	280 ±68 (100%)	130 ±49 (47%)	150 ±54 (54%)
IL-1β (pg/ml)	<15	<15	840 ±100 (100%)	385 ±50 (46%)	490 ±56 (58%)

* Monocyte conditioned media (MOCM) from control and treated monocytes was assayed for IL-1α and IL-1β 48 h after LPS stimulation.

† t_{-6}, Prednisolone was added 6 h before stimulation with LPS.

‡ t_0, Prednisolone was added at the same time as LPS stimulation. Values are expressed as mean ± s.d. for three experiments. Values in parentheses are per cent maximum production.

Table 2. Production of serum amyloid A (SAA) and C-reactive protein (CRP) by HepG2 cells stimulated with monocyte conditioned medium (MOCM) produced in the presence or absence of prednisolone*

	MOCM preparation				
	No LPS		10 μg/ml LPS		
	No prednisolone	1 μM prednisolone	No prednisolone	1 μM prednisolone t_{-6} h†	1 μM prednisolone t_0‡
CRP (ng/ml)	<0·5	<0·5	12 ±3·2 (100%)	2·7 ±1·2 (23%)	3·4 ±1·5 (28%)
SAA (ng/ml)	<2·0	<2·0	318 ±53 (100%)	73 ±45 (23%)	112 ±35 (35%)

* Tissue culture fluid was assayed for SAA and CRP 48 h after addition of MOCM.
† t_{-6}, Prednisolone was added 6 h before stimulation with lipopolysaccharide (LPS).
‡ t_0, Prednisolone was added at the same time as LPS stimulation. Values are expressed as mean ± s.d. for three experiments. Values in parentheses are per cent maximum production.

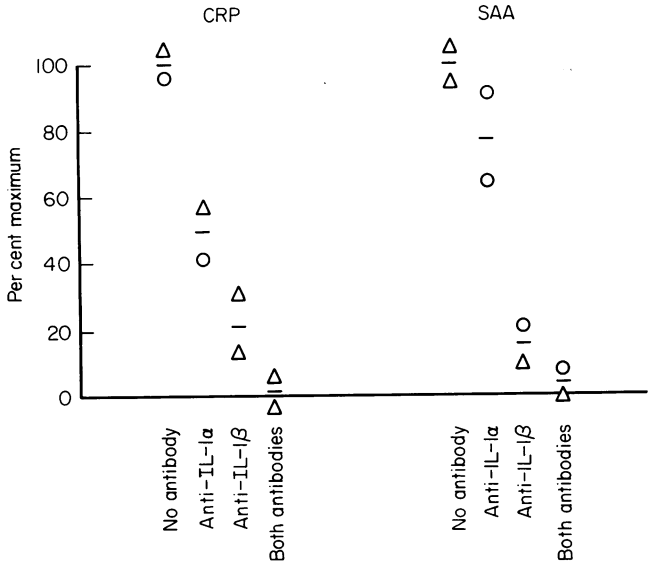

Fig. 1. Effect of treatment of monocyte conditioned media (MOCM) with IL-1α and IL-1β neutralizing antibodies on the induction of serum amyloid A (SAA) and C-reactive protein (CRP) production by HepG2 cells. Monoclonal anti-IL-1α and anti-IL-1β were added to MOCM at 5 μg/ml final concentration. After incubation at 4°C the treated MOCM was used to induce HepG2 cells to produce SAA and CRP. The horizontal lines are the mean values obtained from two experiments; o, actual values.

monocytes which received no LPS stimulation; neither of these induced measurable production of either CRP or SAA.

These data showed that in order to make direct comparisons of the biological activities of the three monocyte-conditioned media ((i) prednisolone added concurrently with LPS; (ii) prednisolone added 6 h before LPS; or (iii) no prednisolone added), it was important to equalize the enhancing effect of prednisolone on SAA production by HepG2 cells. Therefore, each of the filter sterilized MOCM was diluted to 20% in prepared RPMI 1640 media and brought to 1·0 μM prednisolone before testing for their ability to induce CRP and SAA

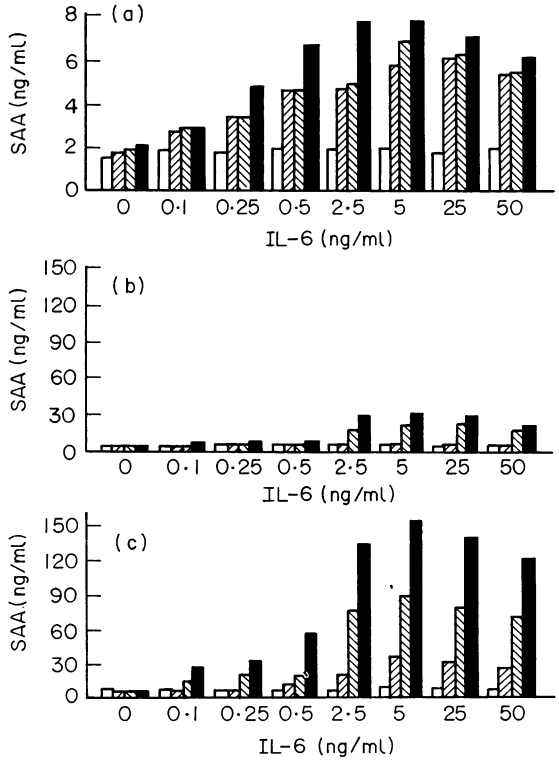

Fig. 2. Production of C-reactive protein (CRP) and serum amyloid A (SAA) by HepG2 cells induced with different concentrations of rhIL-1α, rhIL-6 and prednisolone. Concentrations of rhIL-6 were varied from 0 to 50 ng/ml and concentrations of rhIL-1α were varied from 0 to 25 ng/ml. Values expressed are the means of three experiments. Interexperimental variation was less than 25%. (a) CRP produced in the absence of prednisolone (prednisolone had no effect on CRP production, see text). (b) SAA produced in the absence of prednisolone. (c) SAA produced in the presence of 1·0 μM prednisolone. Concentrations of IL-1α are 0, 1, 5 and 25 ng/ml for □, ■, ▨ and ■ respectively.

production by HepG2 cells. Consistent with measured levels of IL-1α and IL-1β, MOCM from monocytes treated with prednisolone induced significantly less production of CRP and SAA than MOCM obtained from monocytes which were not treated with prednisolone (36% and 28% respectively for SAA and CRP) (Table 2). MOCM obtained from monocytes pretreated with prednisolone 6 h before LPS stimulation induced even less CRP and SAA production (23% for each) by HepG2 cells.

Effect of addition of IL-1α and IL-1β neutralizing antibodies to MOCM on induction of HepG2 cells to produce SAA and CRP

Treatment of MOCM with neutralizing MoAbs to IL-1α and IL-1β reduced its ability to induce the production of both CRP and SAA by HepG2 cells. Individually, each antibody neutralized a portion of the CRP and SAA production activity of MOCM, and when added together, essentially all CRP and SAA production activity was neutralized. This demonstrated that either IL-1α or IL-1β must be present in MOCM in order for HepG2 cells to produce CRP or SAA, and that each was biologically active in our system (Fig. 1).

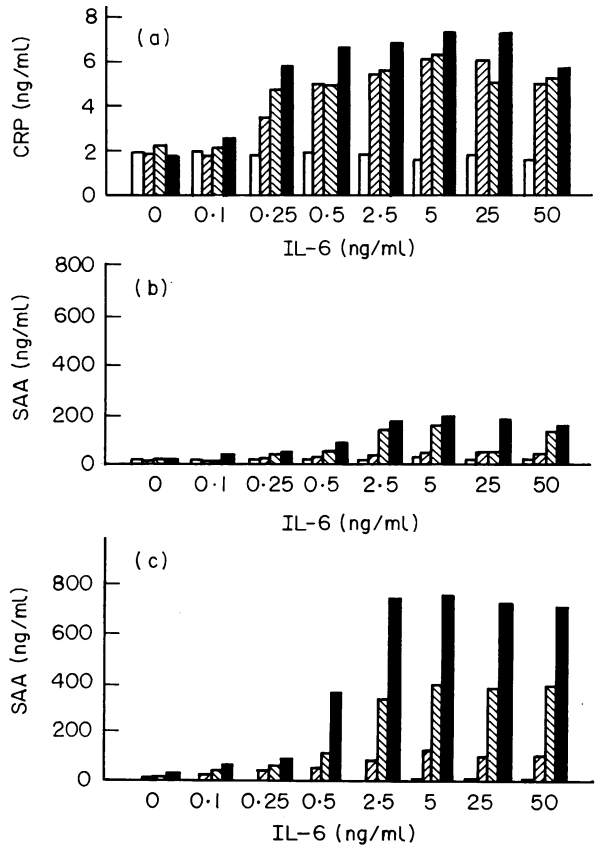

Fig. 3. Production of C-reactive protein (CRP) and serum amyloid A (SAA) by HepG2 cells induced with different concentrations of rhIL-1β, rhIL-6, and prednisolone. Concentrations of rhIL-6 were varied from 0 to 50 ng/ml and concentrations of rhIL-1α were varied from 0 to 25 ng/ml. Values expressed are the means of three experiments. Interexperimental variation was less than 25%. (a) CRP produced in the absence of prednisolone (prednisolone had no effect on CRP production, see text). (b) SAA produced in the absence of prednisolone. (c) SAA produced in the presence of 1·0 μM prednisolone. Concentrations of IL-1β are 0, 1, 5 and 25 ng/ml for □, ■, ▩ and ■ respectively.

Effect of recombinant cytokines and prednisolone on the production of CRP and SAA by HepG2 cells

rhIL-1α, rhIL-6 and prednisolone. Nearly confluent HepG2 cells were stimulated with different concentrations of rhIL-1α and rhIL-6 either in the absence of prednisolone or in the presence of 0·1 μM prednisolone or 1·0 μM prednisolone. CRP production in this system was maximum at 5·0 ng/ml rhIL-6, and 25 ng/ml rhIL-1α (Fig. 2a). Addition of rhIL-6 at concentrations above 5·0 ng/ml caused no further increase in CRP production. Presence of prednisolone had no effect on production of CRP (data not shown).

Maximum SAA production was achieved with rhIL-6 at 5·0 ng/ml and rhIL-1α at 25 ng/ml (Fig. 2b). In contrast to CRP production, SAA production by HepG2 cells was increased three-fold with 0·1 μM prednisolone (data not shown) and up to five-fold with 1·0 μM prednisolone (Fig. 2c). Addition of rhIL-6 at concentrations above 5·0 μM or prednisolone at concentrations above 1·0 μM caused no further increase in SAA production.

rhIL-1β, rhIL-6 and prednisolone. When rhIL-1β was titrated in conjunction with different levels of rhIL-6 to stimulate CRP production by HepG2 cells, maximal activity was found at 5·0 ng/ml of rhIL-6, and 25 ng/ml of rhIL-1β (Fig. 3a). As with rhIL-1α, addition of prednisolone caused no further increase in production of CRP (data not shown).

rhIL-1β and rhIL-6 combined at concentrations of 25 ng/ml of rhIL-1β and 2·5 ng/ml of rhIL-6 caused maximal production of SAA (Fig. 3b), at levels five times higher than the maximum reached with rhIL-1α and rhIL-6 (160 ng/ml as compared with 33 ng/ml). This activity was further enhanced with prednisolone, and reached three-fold higher concentrations with 0·1 μM prednisolone (data not shown) and five-fold higher concentrations with 1·0 μM prednisolone (Fig. 3c).

Effect of rhTNF-α

rhTNF-α was added to HepG2 cells at 750, 200, 50 and 20 U/ml in the presence or absence of rhIL-6 (2·5 ng/ml). rhTNF-α did not induce any measurable production of CRP or SAA in our system. The experiments were repeated with rhTNF-α and rhIL-6 in the presence of 1·0 μM prednisolone with the same results, i.e. no measurable production of CRP or SAA.

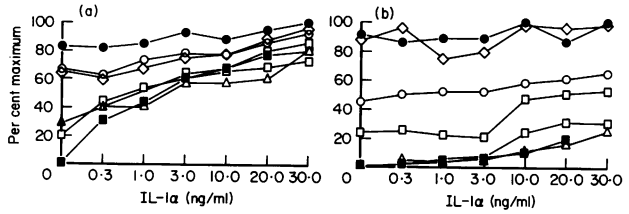

Fig. 4. Production of C-reactive protein (CRP) and serum amyloid A (SAA) by HepG2 cells induced with different concentrations of rhIL-1α and rhIL-1β, and optimal rhIL-6 and prednisolone concentrations. rhIL-1α and rhIL-1β concentrations varied from 0 to 30 ng/ml while rhIL-6 concentration was held constant at 2·5 ng/ml, prednisolone at 1·0 μM. Values expressed are means of per cent maximum production measured in three experiments. Interexperimental variation was less than 25%. (a) CRP produced. (b) SAA produced. ■, Il-1β=0; □, IL-1β=1·0 ng/ml; ○, IL-1β=10·0 ng/ml; ●, IL-1β=30·0 ng/ml; △, IL-1β=0·3 ng/ml; ▲, IL-1β=3·0 ng/ml; ◇, IL-1β=20·0 ng/ml.

Competitive activities of rhIL-1α and rhIL-1β

In the presence of 2·5 ng/ml rhIL-6 and 1·0 μM prednisolone, rhIL-1α and rhIL-1β showed nearly equivalent activity in their ability to induce HepG2 cells to produce CRP (Fig. 4a). Maximum CRP production in this system was achieved with 30 ng/ml each of rhIL-1α and rhIL-1β. rhIL-1α caused CRP to be produced in a dose-dependent fashion so that approximately 70% of maximum production occurred with 30 ng/ml rhIL-1α (Fig. 4a). rhIL-1β under the same conditions induced approximately 80% of maximum production of CRP, also in a dose-dependent manner (Fig. 4a).

In contrast, in the same system rhIL-1α induced only approximately 25% of maximum SAA production in the absence of rhIL-1β, while rhIL-1β at 20 ng/ml and 30 ng/ml induced near maximum (>90%) SAA production in the absence of rhIL-1α. Thus it appeared that for SAA production rhIL-1β was the more potent stimulant, and that high levels of rhIL-1β (≥20 ng/ml) abrogated further SAA production induced by rhIL-1α (Fig. 4b).

DISCUSSION

The findings of this study offer a tenable solution to the disparate production of SAA *versus* CRP observed in CF patients receiving corticosteroid anti-inflammatory therapy [20]. Corticosteroids decreased the production of IL-1α and IL-1β by cultured monocytes, thus decreasing the signal which 'drives' the production of the acute phase proteins by HepG2 cells. However, it appears that corticosteroids can amplify this decreased IL-1 signal to increase the production of SAA several fold, while production of CRP does not appear to have the same amplification mechanism. Castell *et al.* [22] demonstrated that in IL-6-stimulated primary cultures of human hepatocytes the production of SAA was increased six-fold by the presence of 0·1 μM dexamethasone, while the production of CRP was unaffected. Other acute phase proteins were variously affected by dexamethasone; haptoglobin production was doubled, while the production of fibrinogen was unaffected. These reports lend credence to the intimate involvement of corticosteroids in the differential regulation of production of acute phase proteins.

Ghezzi & Sipe [23] showed that when mice were injected with 1 mg dexamethasone 30 min before stimulation with LPS production of SAA was decreased by approximately 70%. This indicated that corticosteroids dampened at least that portion of the acute phase response which resulted in SAA synthesis. However, when IL-1 or TNF was injected into animals that were pretreated with 1 mg dexamethasone SAA production was increased 2·5-fold and 1·5-fold respectively compared with animals that received IL-1 or TNF alone.

The studies conducted with MOCM showed: (i) monocytes stimulated with LPS or killed *Ps. aeruginosa* produced a MOCM that contained both IL-1α and IL-1β, and treatment of the monocytes decreased the production of these cytokines significantly; (ii) MOCM from this system induced the production of both SAA and CRP by HepG2 cells, and part of that induction capability was removed with neutralizing antibody to either IL-1α or IL-1β; and (iii) essentially a complete loss of production of both SAA and CRP was observed when HepG2 cells were stimulated with MOCM in which both IL-1α and IL-1β had been neutralized. When rhTNF-α was added to HepG2 cells with or without rhIL-6 present, no SAA or CRP production was detected. In contrast, Ganapathi *et al.* [12] reported that TNF-α, in the presence of IL-6, induced Hep3B cells to produce SAA, but not CRP, although such production seemed to vary dependent on the batch of TNF-α employed. It is conceivable that a different lot of rhTNF-α might have had activity in our system.

Checkerboard titrations with rhIL-1α and rhIL-6 or rhIL-1β and rhIL-6 at various concentrations of prednisolone revealed, in agreement with recent observations [12, 24], that increased concentrations of either rhIL-1 or rhIL-6 led to an increase in the synthesis of both SAA and CRP by HepG2 cells, so long as a minimal amount of the other cytokine was available. Although rhIL-1β was more active than rhIL-1α in stimulating SAA production they were equally active for inducing the production of CRP.

Maximum SAA production achieved with rhIL-1α was increased by addition of rhIL-1β; however, the opposite was not true. This observation is consistent with the experimental data recently reported by Ghiara *et al.* [10], that HepG2 cells possess two IL-1 receptors, one for IL-1α and one for IL-1β, and the findings of Scapigliati *et al.* [9] that IL-1β is able to displace radiolabelled IL-1α from its receptor, but that IL-1α is unable to displace radiolabelled IL-1β.

The finding that the activities of rhIL-1α and rhIL-1β in stimulating HepG2 cells to produce SAA and CRP were not identical was not unexpected. Raynes *et al.* [4] had previously shown that HUH-7 hepatoma cells, in the presence of 0·1 μM dexamethasone, produced nearly twice as much SAA in response to maximal doses of IL-1α compared with maximal doses of IL-1β. Our findings indicate the opposite effect in HepG2 cells, i.e. that rhIL-1β is the more potent inducer of SAA synthesis. Given the low degree of homology between IL-1α and IL-1β [5], the differences in their synthesis [7, 8, 25], and genetic structure and regulation [6], as well as the existence of two distinct receptors [9, 10], it is probable that different activities will be delineated for IL-1α and IL-1β, even though obviously many overlapping activities exist.

The findings of this study suggest that there may be significant differences in the modulation of different acute phase proteins by different combinations of cytokines and corticosteroids. SAA and CRP levels normally increase concurrently in disease, although Maury & Teppo [14] showed that is not always the pattern followed. The acute phase response is an integrated response involving a number of cytokines (IL-1α, IL-1β, IL-6, TNF, LIF, etc.), hormones such as insulin, and corticosteroids, prostaglandins, etc. These many factors may be produced in different combinations dependent upon the source of initiation, the pathophysiological conditions and modalities of patient therapy. These combinations of factors then set in motion a series of events among which are the differential expression of SAA and CRP.

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